# Effect of Microwave Irradiation for 5, 7 and 10 Minutes toward Risk of Infection on Free Bone Fragment Contaminated with *S. Aureus* and *B. Subtilis*: Experimental Study in Femur of White Rats Wistar Strain

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**ABSTRACT**

**Introduction.** Removal of a large fragment of avascular bone in open fracture case is still controversial. Replacement procedure using *autogenous bone graft* has its limit in volume and the use of *synthetic bone graft* is still expensive.
**Materials and methods.** We conducted an experimental study using white rats Winstar strain. Their femurs were fractured segmentally and stripped from surrounding soft tissue. The bone fragments were then contaminated with *Staphylococcus aureus* and *Bacillus subtulis*. The contaminated fragments received microwave irradiation in different time periode using domestic microwave oven.
**Results.** Microorganism from contaminated bone fragments failed to grow after 7 minutes or more exposure to microwave irradiation.
**Conclusions. S**terilization of a large fragment of avascular bone contaminated with *Staphylococcus aureus* and *Bacillus subtulis* can be achieved using microwave irradiation in domestic microwave oven for certain time period. This method of sterilization of bone fragment is cheap, easily used, and an effective way to process contaminated bone.

**Keywords:** open fracture, large avascular bone fragmen, domestic microwave oven

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# Pengaruh Iradiasi Gelombang Mikro selama 5, 7 dan 10 Menit terhadap Resiko Timbulnya Infeksi pada Fragmen Bebas Tulang Terinfeksi Bakteri *S. Aureus* dan *B. Subtilis*: Sebuah Uji Eksperimental pada Femur Tikus Putih Galur Wistar


## ABSTRAK

**Pendahuluan.** Pembuangan fragmen tulang avaskular yang berukuran besar pada kasus fraktur terbuka masih merupakan suatu kontroversi tindakan penggantian menggunakan *autogenous bone graft* mempunyai keterbatasan jumlah dan penggunaan sintesis *bone graft* terhambat oleh harga yang mahal.

**Bahan dan cara kerja.** Uji eksperimental menggunakan tulang femur dari tikus putih galur Wistar yang dipatahkan secara segmental dan dilepas dari jaringan sekitarnya. Fragmen tersebut kemudian mendapatkan kontaminasi *Staphylococcus aureus* dan *Bacillus subtulis*. Kemudian tulang yang telah terkontaminasi mendapatkan pajanan gelombang mikro menggunakan tungku pemasak domestik selama periode tertentu.

**Hasil.** Pada tulang yang diperiksa menunjukkan tidak didapatkan pertumbuhan bakteri di medium agar pada pajanan gelombang mikro selama 7 menit atau lebih.

**Simpulan.** Penelitian ini menunjukkan bahwa sterilisasi fragmen tulang avaskular berukuran besar yang terkontaminasi *Staphylococcus aureus* dan *Bacillus subtulis* dapat menggunakan gelombang mikro pada tungku pemasak domestik selama periode tertentu. Metode sterilisasi ini murah, mudah digunakan dan efektif untuk tulang yang terkontaminasi.


**Kata kunci:** fraktur terbuka, fragmen tulang avaskular ukuran besar, tungku pemasak domestik

## Introduction

Open fracture is a surgical emergency which can be considered as an partial amputation.[1] In open fracture, there is a connection between bone fragments and soft tissue with external environment. Bacterial contamination from external environment may lead to infection.[2] A century ago, high mortality due to open fracture of long bones caused amputation as a live-saving alternative in open fracture. Even till the beginning of World War I, mortality due to open fracture of femoral bone was still above 70%.[1,2]

After reviewing more than 1000 cases of open fracture in long bones, Gustilo and Anderson create a classification system based on type of injury and wound.[1-3] For illustration, incidence of infected wound connection with degree of soft tissue damage, from only 2% in type I fracture to 10% in type III.[3-5]

Principal of treatment for open fracture in emergency unit is to do general assessment of patient condition and early debridement and irrigation of open wound. To achieve adequate debridement, one must remove all non-vital tissue, like skin, subcutaneous fat tissue, fascia, muscle and free bone fragment. Small avascular free bone fragment should be removed. A heavily contaminated small bone fragment should also be removed because adequate debridement most likely cannot be done. Removal of a large avascular bone fragment is still controversial.[1,7-9] Generally, it is better to remove all avascular bone fragments and replace them with autogenous bone grafting. A retained-avascular bone fragment is the place for bacterial growth and a potential cause of persisten infection after open fracture.

Van Winkle and Neustein,[1] also Kumar, *et al.*,[7] reported attempts to sterilize contaminated large avascular bone fragments in open fractures by washing them in povidone iodine solution, autoclaf, and bathed them in antibiotic solution. Canovas, *et al.*[8] reported a case of open fracture Tibia type IIIB with 12 cm avascular bone fragment. After debridement, the tibial bone fragment was bathed in boiled-normal saline for 20 minutes. Mazurek, *et al.*[9] also reported a case with open fracture femur type IIIA with a large avascular bone fragment. The fragment was bathed several times in chlorhexidine solution and reimplanted 17days post

accident after no more infection in the surrounding soft tissue was observed.

Eventhough there have been many attempts to sterilize free bone fragment in open fracture cases, still there is no standart treatment for contaminated free bone fragment.

Since 18 century ago, researchers wanted to know effect of electromagnetic waves to any living process. Microwave is one part of electromagnetic wave spectrum. Banik, *et al.*,[11] studied the effect of microwave to living-beings and found that microwave affects were proven in all biological-levels, from microbial cells, animals and even human-body systems. Other studies also supported thermal and non-thermal effects of microwave to living-beings.[10-21] Thermal destruction of pathogens was due to heating process during microwave irradiation. If the temperature reached destruction level in pathogen then the cell is destroyed. There are 4 theories describing non-thermal inactivation of pathogen: selective heating, electroporation, membrane-cell destruction, and magnetic field coupling.

In 2000, FDA approved the use of ionizing-radiation to reduce *Salmonella* colonization in raw eggs, but due to concern from consumers, this procedure was not widely used.[15,16] There were several studies that tried to evaluated the use of microwaves in sterilization of food products without damaging the quality.[15-18]

Aziz, *et al.*,[19] from National University Hospital in Singapore, wrote a guadiance book for sterilization of tissue graft and microwaves irradiation was one of the procedure to sterilize contaminated allograft. Ranft, *et al.*,[22] in 1995 conducted a study about the use of microwaves to sterilize bone allograft. That study was then reproduced by Dunsmuir and Gallacher from Glasgow, England, by conducting study using domestic microwave oven to sterilize allograft taken from femoral head and showed that microwave irradiation above 2 minutes can sterilized infected allograft.[23]

Based on those studies, we conducted an experimental study in femoral bone of white-rats Wistar-strain to compare risk of infection that might happen in large free bone fragment Gustilo type III fracture after receiving microwave irradiation for 5, 7 and 10 minutes.

## Materials and methods

This study used thirty two three-month-old white-rats Wistar strain, male, weight approximately 200 g, healthy without any sign of infection on lower extremity. They were allocated into 4 groups, 1 control group and 3 treatment groups.

All rats were anesthesized using intraperitoneal injection of ketamin (40mg/kg) and xylazine (5 mg/kg) for the first operation. The bone fragments were taken from left femur using posterolateral approach. All free bone fragments were bathed in solution containing *Staphylococcus aureus* ATCC 25923 and *Bacillus subtilis* ATCC 6633 with concentration of 1.5 x $10^8$/ $mm^3$ for 15 minutes. The fragments were then irrigated using 100 cc of sterile normal saline. For the control group, all fragments were reimplanted and fixated using K-wire. Treatment group received similar treatment except that the fragments were irradiated using domestic microwave Samsung ME86V-BBH, frequency 2 450 MHz and 800 watt for 5, 7 and 10 minutes prior to reimplantation.

Three days after the first operation, all fragments were extracted and put in transport media and sent to microbiology laboratorium. The fragments were put in incubator for 24-hours and the put in culture media for another 24-hour. All colonies were then examined with gram-stain to identify *S. aureus* and/or *B.subtilis*. Other bacterias were considered as contamination.

The results were analyzed using SPSS for Windows version 16.0. Fisher Exact test will be used to analyze connections between variabels.

## Results

All rats weighted 210 $\pm$ 10 g with femoral length 20 $\pm$ 2 mm. The lengths of the extracted fragments were 7 $\pm$ 1 mm. In control, 7-minutes and 10-minutes groups, 1 rat was dead a day post operatively.

All fragments from control group showed positive cultures with *S.aureus* or *B.subtilis* or both. One fragment showed contamination of *Streoptococcus*. All fragments from 5-minutes group showed positive cultures with *S.aureus* or *B.subtilis* or both. Three fragments showed contamination of *Streoptococcus*.

All fragments from 7-minutes group showed negative cultures. Same results also appeared in all fragments from 10-minutes group.

## Discussions

Lots of studies recommended that to achieve adequate debridement, removal of all non-vital tissues are required, but removal of a large avascular bone fragment is still controversy.[1,4] Limitation in amount of autograft that can be taken and also the high price of allograft make sterilization of contaminated avascular bone fragment a preferred method. Several methods using autoclave had been done but impractical due to cost and time required. Microwave had been use extensively to

sterilize food product without damaging the quality.[3-6] One of the recent study shown that microwave irradiation can sterilize allograft taken from femoral head with only 2-minutes of exposure.

Selection of *S.aureus* and *B.subtilis* as contaminants in this study is deliberate because these bacterias are common pathogen. *S.aureus* is one of the common floras in human skin and one of the bacteria commonly found in infection cases after open fracture. *B.subtilis* is found in soil, water, air and plants. *B.subtilis* produces spore that can withstand harsh enviroment for years. Spore is more difficult to destroy than vegetatif form. Those are the reason to use both bacteria as indicators for effectiveness of microwave sterilization.

Positive cultures in control group showed that both bacterias could grow in avascular fragments similar to open fracture Gustilo type III with avascular fragment. Same results in 5-minutes group showed that exposure of microwave for 5 minutes wass not enough to sterilize avascular bone fragments. Negative cultures in 7-minutes and 10-minutes groups showed that exposure of microwave for 7 minutes or more could sterilize avascular bone fragments using domestic microwave.

This study found that minimum exposure for effective sterilization was 7 minutes, different from the study using allograft from femoral head that showed a minimum of 2 minutes exposure for sterilization. The difference might occur as different part of bone was used. We used diaphysis while other used cancellous bones. Cancellous bones conduct heat better then cortical bones.

**Conclusions**

Sterilization could be done in microwave, through thermal and non-thermal mechanism. Non-thermal mechanism in microwave caused the sterilization to happen in shorter time period, but the exact mechanism needs to be evaluated through another studies.

Further studies need to be performed with larger numbers of bone specimens and larger size of bones to determine if these findings are real. The process of microwave sterilization is cheap and easy to use and can be use to sterilize contaminated free bone fragments in open fracture.

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